

The University of Chicago

PHYSIOLOGY OF THE CILIATE
COLPIDIUM COLPODA. I. THE EFFECT
OF VARIOUS BACTERIA AS FOOD
ON THE DIVISION RATE OF
COLPIDIUM COLPODA

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

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PHYSIOLOGY OF THE CILIATE COLPIDIUM COLPODA. I. THE EFFECT OF VARIOUS BACTERIA AS FOOD ON THE DIVISION RATE OF COLPIDIUM COLPODA¹

(Four figures)

WILLIAM D. BURBANCK²
University of Chicago

ONE trend in contemporary population studies of Protozoa, as recorded in recent extensive reviews (Jahn, 1934; Hammond, 1938; Calkins and Summers, 1941), is toward an analysis of the physiological factors affecting the now familiar Verhulst-Pearl population growth curve, and another is concerned with various growth-promoting and growth-inhibiting substances (Hall, 1941b; Johnson, 1941). As would be expected, these studies have tended to become biochemical in nature. In order to reduce technical difficulties in controlling and repeating experiments, Protozoa have been chosen that will grow in either synthetic inorganic or more complex organic nonparticulate media. These studies have made much progress and are important from both a physiological and an ecological viewpoint; however, in the latter field, on account of their relations to interspecific food relations and food chains, studies of interactions between Protozoa and bacteria must not be overlooked.

The standardization of methods for bacterial-protozoan studies involves certain inherent difficulties. Leslie (1940) has described one method of controlling numbers of food bacteria for *Paramecium*; and he, as well as Cleveland (1928) and Hetherington (1934), has shown that the species and strains of food bacteria are very important.

Until recently, holozoic ciliates could be grown only on bacterial or other living particulate suspensions; now, Taylor and van Wagtendonk (1941) have grown *Colpoda* in a medium which consists of an aqueous extract of brewers' yeast and the plasmoptysate of a marine strain of the bacterium *Pseudomonas fluorescens*. Such work may lead away from bacterial-protozoan studies; yet the use of such media will be valuable for maintaining standard controls and stock cultures for further work on bacterial-protozoan relationships.

When bacteria are used as a source of energy for Protozoa over a period of time in mass cultures, certain internal uncontrolled and unanalyzed changes may take place (Butterfield *et al.*, 1931). In growing large protozoan populations on certain bacteria a division rate may be established, but this rate is actually the net gain. Cutler and Crump (1923) found in their work on *Colpidium*(?) that even in the period of greatest reproduction some of their animals were dying. To obtain a close approximation of the reproduction potential of Protozoa the biotic effects of intraspecific competition between Protozoa, and between bacteria and Protozoa should be removed, and, in any event, must be kept at a minimum. It is extremely difficult to eliminate all such competition, since, as

¹ I am greatly indebted to my adviser, Professor W. C. Allee, for generously expending his time and counsel on many aspects of this problem.

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Kidder and Stuart (1939) have shown, even higher bacteria do multiply in so-called "non-nutrient media."

The isolation small-volume culture technique has been in and out of favor among protozoologists for many years. This technique lends itself ideally to the study of bacterial-protozoan relations, for, if animals are removed from a stock culture in a phase of most rapid division, they may be so maintained in this phase by daily transfers into freshly prepared isolation cultures (Phelps, 1935; Richards, 1941). In this way the environment is not limited, the food possibilities are not exploited in so short a time, and accumulated waste products do not become a problem. On the other hand, the surface-volume relations differ greatly from those in large-volume cultures.

Hetherington (1934) pointed out that the interesting holozoic ciliate *Colpidium colpoda* has been erroneously described as the experimental animal used by several investigators (Peters, 1921; Cutler and Crump, 1923; Butterfield *et al.*, 1931), while Hetherington (1933, 1934) and Chatton and Chatton (1927*a, b*) seem to have actually experimented with this species. As shown by Sandon (1927), this animal is ubiquitous; and it is of further interest because the other good species in the genus, *C. campylum*, can be grown on nonparticulate organic media. Therefore, here is an animal which is at least taxonomically intermediate between the smaller saprozoic forms and the larger holozoic types. Finally, Kidder and Diller (1934) have found no complicated endomixis taking place in the animal; so it, indeed, may be the protozoan par excellence for use in isolation-culture studies.

With this background in mind, *C. colpoda* was used as the experimental animal that was grown on many different monoflorae of bacteria. It should, however, be stated at the outset that this is a survey of a subject which has many ramifications and possibilities. In order to obtain an approximately complete analysis of the interaction between *C. colpoda* and bacteria *x, y, z*, for example, several strains of both the particular protozoan and the particular bacterium should be used simultaneously, and independent and numerous readings should be taken over a relatively long period of time. This is not the task for one person; the methods adopted were, at least, adequate for a one-man reconnaissance only.

MATERIAL AND METHODS

The ciliate *C. colpoda*³ was isolated in the winter of 1939 from a *Parmecium* culture supplied by the General Biological Supply House, Incorporated, of Chicago, Illinois. The culture containing these animals had been originally collected from an unrecorded pond in the Chicago region.

Colpidium colpoda was first placed unwashed into a suspension of the bacterium *Aerobacter aerogenes*. Later a permanent stock was established by washing the animals in sterile 0.3 per cent Difco yeast, according to Parpart's method (Parpart, 1928), and the sterile animals were reinoculated into an *A. aerogenes* suspension made up in sterile artificial pond water and kept in a 125-ml. Erlenmeyer flask. This stock and all experimental and control animals were maintained at $25^{\circ}5 \pm 0^{\circ}5$ C. Unwashed stock animals were transferred to a fresh bacterial suspension once a month throughout the course of the series of experiments, which lasted for over a year.

The experimental work was divided into two parts. First, *C. colpoda* grown on

³ I wish to thank Professor Harold Kirby, Jr., of the University of California, for confirming the identification of this protozoan.

A. aerogenes was used as a control, while thirty-one species of the Eubacteriales, belonging to fifteen genera and six families, were tested as food organisms. Second, the same procedure was repeated with the control animal grown on *P. fluorescens*.

As this work is a preliminary phase of an ecological laboratory study of the population of *C. colpoda*, only aquatic or ubiquitous bacteria were used. Of the fifteen genera studied, eight were found to be present in fish-conditioned water used in this laboratory.⁴ The only other genus found in this water was *Achromobacterium*, which was not worked with because of the uncertainty of the taxonomy in this group. *Bergey's Manual of Determinative Bacteriology* (1939) was the taxonomic authority in this work; and, when at all possible, an attempt was made to use the type species of any particular genus as one of the organisms tested in that genus. The bacteria used were obtained from the collection of cultures of the department of bacteriology of the University of Chicago and from the American Type Culture Collection in Washington, D.C.

All bacteria used as food for *C. colpoda* were first transferred to veal-infusion slants and incubated at their optimal temperatures for growth. After 24 hours each slant was washed with 1 ml. of sterile artificial pond water, and growths from three slants were transferred to Kolle flasks containing veal-infusion agar. These, in turn, were incubated for 24 hours at the correct temperatures; then, 5 ml. of sterile artificial pond water were added, and this water was swirled for several minutes with the flask in a horizontal position. No mechanical means were employed to loosen the bacterial growths, with the exception of growths of *Micrococcus conglomeratus* and *Bacillus mycoides*, which adhered tenaciously to the surface of the medium. When a suspension of the bacteria had been obtained, it was added to 25 ml. of sterile artificial pond water. A check of the viable bacteria was made by the dilution method. After this count a stock suspension of the bacteria was prepared such that it approximated the arbitrary number of 150,000,000 viable bacteria per milliliter. These stocks were checked immediately by the dilution method of the beginning of the 10-day period and again at the end of the experiment. The bacterial stock suspensions, which were generally between 90 and 100 ml., were kept for the 10-day period in a cold room at about 6° C.; and daily 0.2 ml. of the suspensions were dispensed to each experimental and control line. The final count showed that the number of viable bacteria remained constant over the 10-day period. The vibrios micrococci, and several spore-formers had to be used at lower dilutions than the one stated above, since with these forms such magnitudes of viable bacteria were so opaque that the Protozoa could not be seen.

At the beginning of a series of experiments single animals were taken from the stock culture and placed in 0.2 ml. of *A. aerogenes* suspension, which had been pipetted into a sterile hard-glass watch glass placed in a sterile Petri dish containing a few milliliters of distilled water. An attempt was made to take animals from the stock culture at the phase of most rapid division or, if this was not possible, to subculture them until they were dividing at a maximum division rate. In the first experimental run of both the *P. fluorescens* and the *A. aerogenes* series, experimental animals were taken from mass cultures; and after that all experimental animals were taken from isolation-culture controls of a previous run. It will thus be seen that the animals used in each series were from cultures grown on the same bacterium that was used in growing the controls for that particular series. Because of unavoidable interruptions, two exceptions existed in the *A. aerogenes* series: animals had to be kept in mass culture during the months of October

⁴ By private communication with Mrs. Murvel Garner.

and January, when no experiments were run; but, as mentioned above, these animals were not used for experiments in November and February, respectively, until they were again dividing at a high rate.

When an isolated animal had divided several times, one or several animals were subcultured singly on *A. aerogenes* as unwashed controls, and the rest were washed with five washes of sterile 0.3 per cent yeast extract. After remaining in the fifth wash for 3-5 hours the experimental animals were again washed five times (Parpart's method). Animals were taken from the last wash (No. 10) and placed in the desired bacterial suspensions. The last wash medium was incubated for 48 hours as a test of sterility, and only animals were used that proved to be sterile according to this test. There was found to be no statistical difference between division rates of washed and unwashed animals.

After the experimental and control animals had increased to at least four individuals, these animals were then used to start four lines, which were grown in isolation cultures for a 10-day period with daily subcultures of one animal per line. In subculturing animals a consistent attempt was made to take at random the animals to be transferred. Dividing individuals, or those having just divided, were not used if other individuals not in these stages were available. During the 10-day period, 24-hour counts were made with the aid of a dissecting microscope and a mouth-controlled micropipette. In all individual experiments actual counts, not estimations, of the animals produced in 24 hours were made.

With a few exceptions (see Figs. 1 and 2) the experimental unit used during the 10-day experimental period consisted of two experimental sets of four lines each and one four-line control, all of which were kept in one large crystallizing dish. The cultures were prevented from evaporating by the moisture from several thicknesses of water-saturated filter paper placed in the bottom of the chamber. All the watch glasses containing Protozoa were placed in a regular and consistent order on a circular metal rack and kept from slipping from position by rubber washers attached to the top of the rack. When the Protozoa were to be transferred, the watch glasses were placed in sterile Petri dishes to facilitate handling.

To preclude any contamination while making transfers, a dustproof box⁵ was used. This was fitted with a transparent cellulose-acetate spectacle-shaped shield (Plastocele) attached to an upper central opening of the box with oiled silk. The shield fitted over the oculars of a dissecting microscope, making it possible to handle the animals within the box with a minimum danger of contamination. The top and back of the box were glass, and the front and the side inlets were closed with oiled silk "zipper" curtains. During the experimental period the box was washed several times with 70 per cent alcohol, and the dust-free dissecting microscope was used only for transfers. When not in the transfer box, the microscope was kept under a bell jar. Animals were transferred with small sterile mouth-controlled micropipettes. Before the operator's hands were introduced into the box, they were always washed with 70 per cent alcohol.

Hydrogen-ion concentration tests were made on the experimental and control bacterial suspensions with the Hellige method before, during, and after an experimental period. All bacterial suspensions used were made up in unbuffered, sterile artificial pond water, which was developed in this laboratory by Allee *et al.* (1934) and contained per liter:

⁵ I wish to thank my father, the Rev. Mr. G. G. Burbanck, for the construction of the dustproof transfer box.

100 mg. $\text{CaCl}_2 \cdot 4\text{H}_2\text{O}$
50 mg. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

50 mg. K_2SO_4
50 mg. NaNO_3

All water used for making up the artificial pond water was Pyrex-redistilled water with an arbitrary lower limit of goodness of 2.00×10^{-6} reciprocal ohms.

As a check for bacterial contamination, Gram's stains were run on the bacterial suspensions before they were used and from the last 0.2-ml. cultures in which the experimental and control animals had been living. In only one case out of sixty was any con-

TABLE 1

RECORD OF A TYPICAL EXPERIMENTAL UNIT

I. <i>Salmonella paratyphi</i> A.												II. <i>S. schottmulleri</i>											
EXPERIMENTAL SUBCULTURES	MAY										MEAN DIV.	EXPERIMENTAL SUBCULTURES	MAY										MEAN DIV.
	9	10	11	12	13	14	15	16	17	18			9	10	11	12	13	14	15	16	17	18	
A.....	4	4	4	6	4	6	4	3	4	6	2.1340	E.....	4	8	6	8	8	4	6	3	7	4	2.4562
B.....	6	4	5	7	4	7	4	5	5	4	2.3166	F.....	7	8	6	4	4	5	8	7	7	4	2.5329
C.....	2	8	4	7	4	4	4	5	4	4	2.1129	G.....	4	8	4	5	8	3	6	5	4	4	2.2814
D.....	4	2	5	4	7	4	4	4	4	4	2.0129	H.....	8	6	4	4	4	4	6	4	6	4	2.2755
Mean of means											2.1441	Mean of means											2.3865

III. <i>P. fluorescens</i>											
CONTROL SUBCULTURES	MAY										MEAN DIV.
	9	10	11	12	13	14	15	16	17	18	
I.....	8	8	6	8	7	4	5	3/k	5	8	2.5621
J.....	8	8	8	14	4	8	8	3/k	4	8	2.7392
K.....	7	8	8	11	4	7	4	8	4	8	2.7074
L.....	8	8	8	8	8	8	7	8	8	8	2.9807
Mean of means											2.7474

tamination discovered. The results from this experiment were discarded, and the work was repeated. The dilution plates used for checking viable bacteria were also observed for contaminating colonies. For sterilization and handling of cultures and media the usual bacteriological techniques were employed, and methods for wrapping glassware and sterilization followed Buchsbaum and Loosli (1936). All bacterial media used were supplied by the department of bacteriology of the University of Chicago.

PRESENTATION OF DATA

Table 1 shows the results from a typical experimental run. It will be seen that the procedures follow, in a modified form, those Calkins (1933) employed in his studies on protozoan vitality. Fewer lines were used in this work, and no running record of the

number of generations was kept. Parts I and II of the table show the divisions of *C. colpoda* when feeding on *Salmonella paratyphi* A. and *S. schottmulleri*, respectively, while Part III gives the results on the control organism, *P. fluorescens*. The different protozoan subcultures are designated by letters of the alphabet. On May 16 the two subcultures, I and J, contained three animals each which were abnormal for some unknown reason. Since experience had shown that subculturing such abnormal animals would unduly affect the mean of the means for the whole run, as these animals would either die or remain undividing for days, whenever such abnormal individuals appeared, or if there had been a death on the previous day, an individual was taken from another line to keep four lines going for the whole 10 days. It was necessary to follow such a procedure in only 10 per cent of all of the experimental and control runs.

In analyzing the data the actual number of animals produced in 24 hours had to be converted into the equivalent number of divisions. Since the reproduction of *C. colpoda* was exponential, the conversion was made by using the logarithm of the number of individuals per day per line to the base 2. Then the means were obtained which appear at the end of each line in Table 1. The mean of these division rates is given below the four averages mentioned and is the mean division rate for four lines of *C. colpoda* for a 10-day period.

The crux of this problem is whether different species of bacteria, when used as food for *C. colpoda*, significantly change its division rate. Since Protozoa, as with all biological systems, and unlike chemically pure reagents, are highly variable, it is necessary in such problems to accompany experimental tests by a standardized control with which cross-comparisons can be made. In the past such comparisons have usually been made merely by inspection of the mean division rates without subjecting these means to further statistical analyses. With small samples it is generally realized that the statistical significance is limited to the set of data which is being analyzed. In the present instance it is recognized that the experimental tests on any given bacterial species have necessarily been limited in number and that further testing might conceivably change the trends which are indicated in the present work. Even so, the use of statistical analysis of the significance of the differences between mean values, if not overgeneralized, serves as an advance over estimates of significance based upon examination of the means alone.

In the analysis of the present work the method used to test the statistical significance of the observed differences was essentially that developed by "Student" (1925) and by R. A. Fisher (1925).⁶ A conservative method was used in the calculation of the *P*-values. As described above, mean division rates were obtained for both experimental and control

⁶ Treating the means of four experimental and four control lines provisionally as one homogeneous population, the following formula for *t* may be used:

$$t = \frac{(\bar{v}_1 - \bar{v}_2)\sqrt{N}}{\sigma_{\text{Total}}},$$

when

$$\sigma_{\text{Total}} = \sqrt{\frac{\sum v^2 - \frac{(\sum \bar{v})^2}{N}}{N - 1}},$$

$\bar{v}_1$ is the mean of means of the control, and $\bar{v}_2$ is the mean of means of the experiment. $\bar{v}$ is the grand mean, and *v* is the number of a single control or experimental line. *N* is the number of control and experimental lines.

TABLE 2
DIVISION RATES OF *C. colpoda* GROWN ON THIRTY-ONE
SPECIES OF THE EUBACTERIALES

Bacterial Species Used	Mean 10-Day Division Rate for Four Lines	P
I. Rhizobiaceae:		
<i>Chromobacterium violaceum</i>	Toxic	
1. <i>Ch. viscosum</i>	2.1080	0.0008
<i>P. fluorescens</i> *.....	3.0845	
2. <i>Alcaligenes fecalis</i>	1.5439	0.0016
<i>P. fluorescens</i>	2.7424	
3. <i>Al. viscosus</i>	2.5467	0.0106
II. Pseudomonadaceae:		
4. <i>Vibrio comma</i> (survived 5 days).....	0.3000	0.0012
<i>P. fluorescens</i>	3.0161	
5. <i>V. metchnikovii</i> (survived 4 days)....	0.2500	0.0012
<i>P. fluorescens</i>	3.0315	
6. <i>P. aeruginosa</i>	1.0708	0.0012
<i>P. fluorescens</i>	2.5578	
7. <i>P. fluorescens</i>	2.7500	0.0182
III. Micrococcaceae:		
8. <i>M. luteus</i>	0.3646	0.0012
<i>P. fluorescens</i>	2.9876	
9. <i>M. conglomeratus</i>	1.3378	0.0012
<i>P. fluorescens</i>	3.0922	
10. <i>Staphylococcus aureus</i>	1.8825	0.0017
11. <i>St. citreus</i>	0.4250	0.0012
12. <i>St. albus</i>	1.8844	0.0017
<i>P. fluorescens</i>	2.9224	
13. <i>Sarcina flava</i>	1.2085	0.0012
<i>P. fluorescens</i>	3.0922	
14. <i>Sar. lutea</i>	0.3875	0.0012
IV. Enterobacteriaceae:		
15. <i>Escherichia coli</i>	2.1414	0.0016
<i>P. fluorescens</i>	2.8094	
16. <i>A. aerogenes</i>	2.9322	0.0622
<i>P. fluorescens</i>	2.8615	
17. <i>A. cloacae</i>	2.3784	0.0016
18. <i>Serratia marcescens</i>	2.4495	0.0334
<i>P. fluorescens</i>	2.5941	
19. <i>Ser. plymouthensis</i>	2.7848	0.0228

* As in this case, whenever *P. fluorescens* appears unnumbered, it is the basic food organism for control *Colpidium*.

TABLE 2—Continued

Bacterial Species Used	Mean 10-Day Division Rate for Four Lines	P
20. <i>Proteus vulgaris</i>	2.7315	0.0196
<i>P. fluorescens</i>	2.9988	
21. <i>Pr. mirabilis</i>	2.1077	0.0016
22. <i>S. schottmulleri</i>	2.3865	0.0038
<i>P. fluorescens</i>	2.7474	
23. <i>S. paratyphi</i> A.	2.1441	0.0034
24. <i>Eberthella typhosa</i>	1.8321	0.0016
<i>P. fluorescens</i>	2.8094	
V. Bacteriaceae:		
25. <i>Flavobacterium synxanthum</i>	1.5015	0.0020
<i>P. fluorescens</i>	2.4471	
26. <i>F. acetylicum</i>	1.7829	0.0030
VI. Bacillaceae:		
27. <i>B. subtilis</i>	0.0750	0.0012
<i>P. fluorescens</i>	2.6093	
28. <i>B. mesentericus</i> (survived only 1 day after several unsuccessful attempts to start it).....		
29. <i>B. mycoides</i>	0.2750	0.0012
<i>P. fluorescens</i>	2.6298	
30. <i>B. megatherium</i>	1.3646	0.0012

lines; then the four mean division rates of *C. colpoda* grown on a given bacterium and the means from the four accompanying control lines were provisionally treated statistically as one homogeneous population. If, then, the difference between the means of the experimental and the control lines gave, by the *t*-test, a *P*-value greater than the 0.05 level of significance, it was assumed that there was no significant difference between the experimental and the control division rates. However, if the *P*-value was smaller than 0.05, the difference of the division rates between *C. colpoda* grown on the experimental bacterium and the control species (*A. aerogenes* or *P. fluorescens*) was accepted as a real one, i.e., the control and the experimental animals statistically belonged to different populations; and, under the conditions tested, this difference was considered to be due to some quality or qualities of the experimental bacterium. This difference was even apparent, in all but a few cases, when the level of significance was changed from 0.05 to the much lower, but sometimes employed, level of 0.01.⁷

To facilitate the understanding of the experimental results, Table 2 has been arranged with the experimental bacteria grouped according to families. For ready reference the experimental bacteria were numbered in Arabic and the families with Roman numerals. In this series of experiments *Colpidium* grown on *P. fluorescens* was the control. When the experimental animal was grown on a single bacterial species of a genus, that fact was recorded by a single name entered into the table, with its control placed directly below

⁷ I am indebted to Dr. Earl L. Green, of Ohio State University, for suggestions on handling the statistics used in this paper.

it; also, the two species in two genera (*Vibrio* and *Micrococcus*) have their controls below each species name. Since three staphylococci were run at one time, their control is directly below their several names. With these exceptions, the name and record of each control was placed between the names and records of each of its experimental bacteria, since they were run at the same time as shown in Table 1. The mean 10-day division rates of *C. colpoda* grown in experimental and control bacterial suspensions are found immediately to the right of each bacterial name, while the *P*-, or probability, value in every case refers to a simple statistical comparison of each experimental performance of *Colpidium* with its own control. It can be ascertained from Table 2 that the division rates of the experimental animal were, in all but a few cases, radically different from their control; i.e., *P* is smaller than the arbitrary value of 0.05.

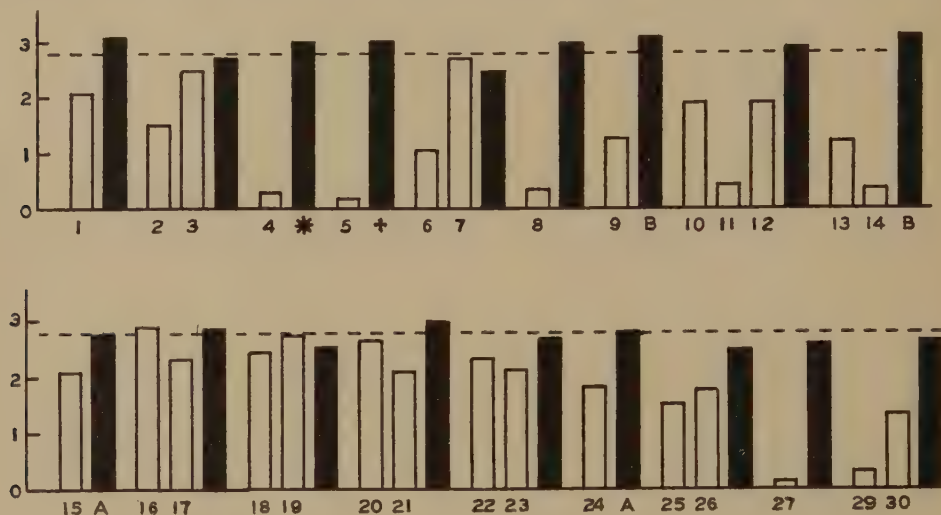


FIG. 1.—The white bars show the average 10-day division rates of *C. colpoda* grown on different species of bacteria; the black ones show the accompanying rates when grown on *P. fluorescens*. Ordinates show division rates; abscissae give the numbers assigned to bacteria in Table 2. The broken line gives the mean for all controls. Controls marked by the same letters are identical. *Five days. +Four days.

The relationships of *C. colpoda* grown on different bacterial species are more strikingly set forth in Figure 1 than in tabular columns of figures. Although all experiments could not, of course, be carried on at one time, a comparison of the black control bars with the broken line indicates that the division rates of the controls were quite constant. In the case of the vibrios, their controls had two of the three highest division rates, so that the poor showings of *Colpidium* on these bacteria were certainly not due to weak experimental animals, since the control and experimentals came from the same stock. A comparison of each pair of white experimental bars indicates that, besides differing from their controls, the different species of bacteria in the same genus supported division rates of *C. colpoda* that were different.

As stated earlier, *C. colpoda* was also grown on a series of thirty-one species of bacteria with *A. aerogenes* as food for the control animals. Because the resulting data were handled in the same manner as represented in Table 2, they were not included in a sepa-

rate table but are given in Figures 2 and 3, which, together, adequately present the experimental results.

Certain significant comparative data can be obtained by careful examination of Figures 1 and 2. On *P. fluorescens* (Fig. 1) the control ciliates had an average division rate that was much higher than when they were grown on *A. aerogenes*. Also, the rate of growth on the controls was more uniform on the former, as indicated by a comparison of the broken lines with the black bars in Figures 1 and 2. The black control bars are more numerous in Figure 2 than in Figure 1, owing to the fact that more bacteria were tested singly against their *A. aerogenes* controls, since all the experimental bacterial cultures were not available at the beginning of the *A. aerogenes*-controlled experiments. The highest rate of division among the controls shown in Figure 2 is that of the control for *Ch. viscosum*. This division rate is higher than that of any for *C. colpoda* grown on

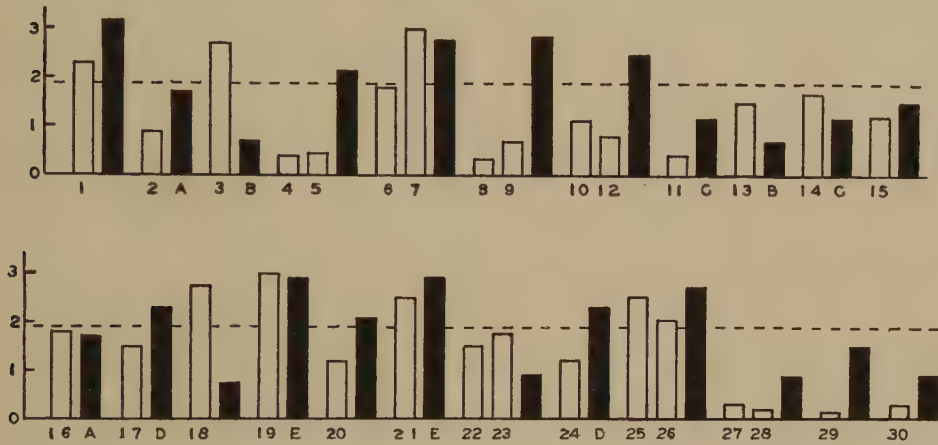


FIG. 2.—The white bars show the average 10-day division rates of *C. colpoda* grown on different species of bacteria; the black ones show the accompanying rates when grown on *A. aerogenes*. Ordinates show division rates; abscissae give the numbers assigned to bacteria in Table 2. The broken line gives the mean of all controls. Controls marked by the same letters are identical.

P. fluorescens. In spite of the unusually high division rates which *A. aerogenes* was capable of supporting, the average division rate of these control animals was lower than that for those grown on *P. fluorescens*. In general, the animals which had been taken from *P. fluorescens* suspension divided at a higher rate but did not necessarily yield experimental animals with higher experimental division rates than those taken from *A. aerogenes* cultures. For example, *Ch. viscosum* (3), *Sar. lutea* (14), and *Ser. marcescens* (18) in Figure 2 all have controls which have lower division rates than those of the corresponding experiments shown in Figure 1; nevertheless, the foregoing three experimental bacteria supported higher division rates of *C. colpoda* than the same bacteria in Figure 1. *Bacillus mesentericus* appears as an experimental bacterium in Figure 2 but not in Figure 1, because it was unable to support *Colpidium* taken from *P. fluorescens* stocks. The two species of vibrios were able to support the animals taken from *P. fluorescens* stocks for only a few days, although animals obtained from *A. aerogenes* suspensions survived on the vibrios for the entire 10-day period. In both Figure 1 and Figure 2 it can

be seen that different species of bacteria belonging to the same genus differ in their ability to support *C. colpoda*, whether obtained from *P. fluorescens* or from *A. aerogenes* stocks. This point and others which concern the comparison of the data in Figures 1 and 2 are discussed in more detail in the following paragraphs.

Although Figure 3 does not help to explain the erratic division rates of *Colpidium* grown on *A. aerogenes*, its chronological arrangement does clearly illustrate a rather extensive period of lowered reproduction. The division rate of *C. colpoda* grown on *A. aerogenes* as the control bacterium started in August, 1940, with a fairly high rate, which continued near or above the average through December. In February, however, division rates became low, increased again in late March, and remained high until the end of

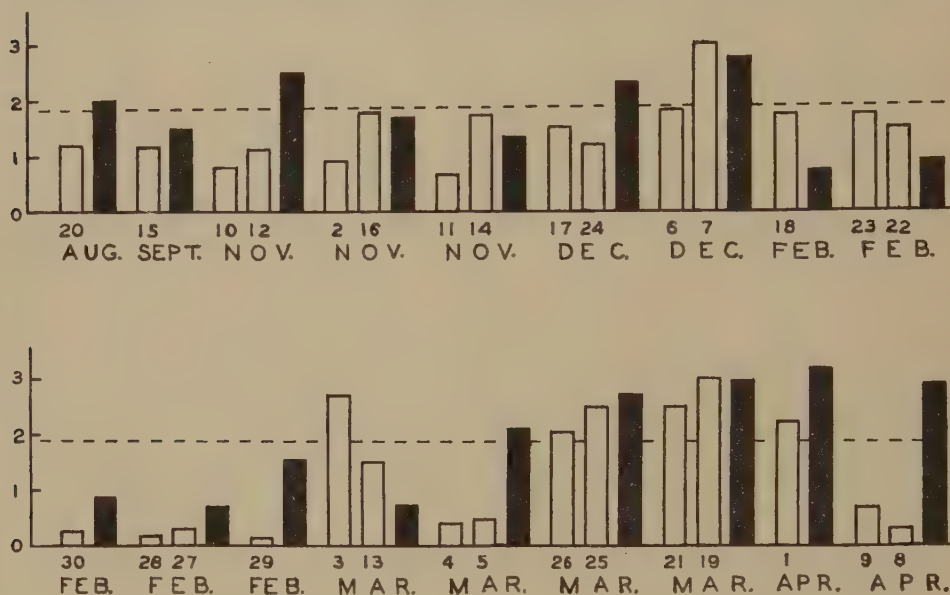


FIG. 3.—A chronological arrangement of Fig. 2

that whole experimental series. That these effects were caused by *A. aerogenes* rather than by some internal change in the animal itself is shown, in Figure 3, by the fairly high division rates of *Colpidium* grown on *Al. viscosus* (3) and *Ser. marcescens* (18), which had division rates more than twice as great as those of their control animals, although both control and experimental animals had recently come from the same culture. There is no explanation for the remarkable March and April recovery of *C. colpoda* controls grown on *A. aerogenes*. However, it was noted that *A. aerogenes* underwent an autoagglutination that regularly appeared on the fifth or sixth day in the 90-ml. stocks kept at 6° C. Hetherington (1934) believed that this in some way caused the lowered division rates. As agglutination consistently occurred in all stock suspensions, this phenomenon probably has no effect on these "epidemics," as the periods of reduced rates of reproduction were called by Hetherington.

During the runs numbered 10, 11, 12, 16, and 17 in Figure 3 *Colpidium* in the control and occasionally in the experiment produced one or two aberrant individuals per day

per line. Only normal animals were subcultured, but still a few abnormal types were produced throughout these runs. It appeared that these bizarre forms were a result of some upset in the time of division, for perfectly formed doubles were found with the posteroanterior ends attached. From the violent pulling that these double monsters exhibited, it was evident that, had they pulled apart, one would have had a mangled posterior and the other a misshapen anterior end. These peculiar malformations were noted in only the particular experiments here cited, and the conditions for these particular tests were the same as for all other experiments, as far as it was possible to determine. No such performances of *Colpidium* were noted at all in the series of experiments based on *P. fluorescens*.

It will be noted in Table 2 that *P. fluorescens* (7) contained washed *C. colpoda* which had an average division rate for a 10-day period of 2.7500, and the control had a *significantly different* division rate of 2.5578. Although this is contrary to an earlier statement (p. 345), this discrepancy can readily be explained. During the last 9 days of the experiment washed versus unwashed animals were compared, and no significant difference was found. In addition, the controls for *E. coli* (15) and *A. cloacae* (17), which were run at the same time, were not significantly different from the washed *C. colpoda* grown in the bacterial suspension common to these three, i.e., *P. fluorescens*. It was found upon reference to laboratory notes that the lower division rate of 2.5578 was due to the few divisions of the control animals on the first day; and this, in turn, was found to have been caused by the slightly different times of divisions of the control and experimental animals before the start of the experimental run. The four animals for the control were taken from a culture which contained seven individuals, while those used for the experiment were taken from one which contained eight animals. This, when interpreted, means that the control animals had not yet finished their third division, while the experimental animals, which had come from one washed animal on the previous day, were beginning on their fourth. Therefore, it may be concluded that the earlier statement is justified in supposing that washing animals causes no significant difference in the subsequent division rate. Fortunately, this case is unique, but it does bear witness to the sensitivity of the system. Otherwise, in experiments in which *P. fluorescens* was used as the control and basic food organism (Table 2), only *C. colpoda* grown on *A. aerogenes* and those grown on *Ser. plymouthensis* had division rates which were higher than those of the accompanying control series. The difference in the case of the former falls above ($P = 0.0622$), and in the case of the latter below ($P = 0.0228$), the stated upper limit of statistical significance. *Serratia marcescens* and *A. cloacae* supported division rates of *C. colpoda* which were somewhat less than that of their accompanying control with $P = 0.0334$ and $P = 0.0016$, respectively.

When the mean 10-day division rates of *C. colpoda* given in Table 2 were tested statistically (Table 3), it was found that, with the exception of *St. aureus* and *St. albus* (Table 3), different bacterial species of the same genus had significantly different growth-promoting powers. If these data are verified by a more extensive series of tests, this would open an interesting line of study for students of speciation.

In the series in which *A. aerogenes* was the basic food and the food organism for the control series (see Fig. 2), several species supported significantly faster division rates than those shown in the accompanying controls; they were as shown on page 355. All the other experimental bacteria supported fewer divisions of *C. colpoda* than those shown in the accompanying controls. *Serratia plymouthensis* was the only experimental

TABLE 3

DIVISION RATES OF *C. colpoda* GROWN ON DIFFERENT
BACTERIAL SPECIES OF THE SAME GENUS

Bacterial Species Used	Mean 10-Day Division Rate for Four Lines	P
I. Rhizobiaceae:		
1. <i>Ch. violaceum</i>	Toxic }	0.0020
2. <i>Ch. viscosum</i>	2.1080 }	
3. <i>Al. fecalis</i>	1.5439 }	
4. <i>Al. viscosus</i>	2.5467 }	
II. Pseudomonadaceae:		
5. <i>P. aeruginosa</i>	1.0708 }	0.0012
6. <i>P. fluorescens</i>	2.7500 }	
III. Micrococcaceae:		
7. <i>M. luteus</i>	0.3646 }	0.0012
8. <i>M. conglomeratus</i>	1.3378 }	
9. <i>St. aureus</i>	1.8825 }	0.9952
10. <i>St. albus</i>	1.8844 }	
9. <i>St. aureus</i>	1.8825 }	0.0012
11. <i>St. citreus</i>	0.4250 }	
10. <i>St. albus</i>	1.8844 }	0.0012
11. <i>St. citreus</i>	0.4250 }	
12. <i>Sar. flava</i>	1.2085 }	0.0012
13. <i>Sar. lutea</i>	0.3875 }	
IV. Enterobacteraceae:		
14. <i>A. aerogenes</i>	2.9322 }	0.0014
15. <i>A. cloacae</i>	2.3784 }	
16. <i>Ser. marcescens</i>	2.4405 }	0.0024
17. <i>Ser. plymouthensis</i>	2.7848 }	
18. <i>Pr. vulgaris</i>	2.7315 }	0.0020
19. <i>Pr. mirabilis</i>	2.1077 }	
20. <i>S. schottmulleri</i>	2.3865 }	0.0058
21. <i>S. paratyphi</i> A.....	2.1441 }	
V. Bacteriaceae:		
22. <i>F. synxanthum</i>	1.5015 }	0.0196
23. <i>F. acetylicum</i>	1.7829 }	
VI. Bacillaceae:		
24. <i>B. subtilis</i>	0.0750 }	No growth }
25. <i>B. mesentericus</i>	No growth }	
26. <i>B. mycoides</i>	0.2750 }	0.0014
27. <i>B. megatherium</i>	1.3646 }	

bacterium to support a division rate of *Colpidium* approaching its control, and even in the small difference in mean division rate of 0.1085, *P* equaled 0.0204. As seen in Figure 2, all other experiments and accompanying controls were quite different, and calculations showed very low *P*-values, which means that the differences, as observed, had high statistical significance.

Bacteria	Difference between Experimental and Control Means
<i>Al. viscosus</i> (3).....	1.9367
<i>P. fluorescens</i> (7).....	0.2295
<i>Sar. flava</i> (13).....	0.7585
<i>Sar. lutea</i> (14).....	0.5317
<i>Ser. marcescens</i> (18).....	0.6749
<i>Ser. plymouthensis</i> (19).....	0.1085
<i>S. schottmulleri</i> (22).....	0.5683
<i>S. paratyphi</i> A. (23).....	0.8707

From Figures 1 and 2 certain further relationships may be abstracted. In both figures the broken line represents the average division rate of *C. colpoda* on its respective control bacterium. The average for *P. fluorescens* was 2.7917 ± 0.0534 (standard error) and for *A. aerogenes* was 1.8597 ± 0.1961 . These two results should be taken seriously, since the first is based on fourteen separate experiments of four different lines each, and the

TABLE 4
RELATIONSHIPS OF TWO OR MORE BACTERIAL SPECIES OF A GENUS DE-
TERMINED BY THEIR EFFECT ON DIVISION RATE OF *C. colpoda*
WHEN TAKEN FROM TWO DIFFERENT BACTERIAL FOODS

Relation Similar	Relation Reversed	Relation Uncertain
<i>Alcaligenes</i> <i>Pseudomonas</i> <i>Micrococcus</i> <i>Staphylococcus</i> <i>aureus</i> vs. <i>citreus</i> <i>albus</i> vs. <i>citreus</i> <i>Aerobacter</i> <i>Serratia</i> <i>Bacillus</i> <i>megatherium</i> vs. <i>mycoides</i>	<i>Sarcina</i> <i>Proteus</i> <i>Salmonella</i> <i>Flavobacterium</i>	<i>Staphylococcus</i> <i>aureus</i> vs. <i>albus</i> <i>Bacillus</i> <i>subtilis</i> vs. <i>mesentericus</i>

second on eighteen such experiments. Using the method described above for testing probabilities, these two averages are significantly different, with a *P*-value of 0.0000.

Omitting the performances of *Colpidium* on the two species of *Vibrio* and *B. mesentericus* because of its inability to survive for 10 days on these bacteria when taken from *P. fluorescens*, the experimental series (Figs. 1 and 2) were treated statistically, so that the division rates for *C. colpoda* grown on the same species of experimental bacteria in these two series could be compared. For this purpose the two performances of *Colpidium* on each of the twenty-seven bacteria were paired, and Student's method of paired com-

parisons was applied. It was found that, in the cases tested, *Colpidium* taken from *P. fluorescens* and grown on suspensions of different experimental bacteria was significantly different ($P = 0.0000$) from experimental animals taken from *A. aerogenes* cultures. It is interesting to note that this difference was not due to a consistently low or high division rate of animals taken from either of the control bacterial suspensions, for, of these twenty-seven pairs of experiments, animals taken from *P. fluorescens* had higher division rates than those grown on suspensions of *A. aerogenes* for fourteen pairs, while the reverse was true for thirteen pairs when *C. colpoda* was taken from *A. aerogenes*.

With regard to specific differences in a genus of bacteria, in most cases, if *C. colpoda* had a higher division rate on one of two species when taken from *P. fluorescens*, this same relationship held when it was taken from *A. aerogenes*. In a few cases this relationship did not hold. The relationships for all sixty experiments of *C. colpoda* taken from *P. fluorescens* and *A. aerogenes* and grown on the same experimental bacteria are shown in Table 4.

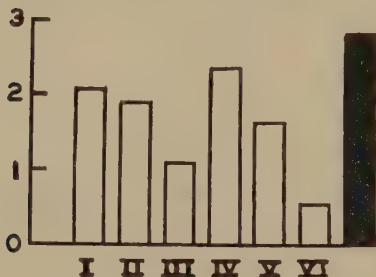


FIG. 4.—The white bars show the average 10-day division rates of *C. colpoda* grown on twenty-seven species of bacteria arranged as family averages. The black bar is the average division rate of the controls for all six families grown on *P. fluorescens*. Ordinates show division rates; abscissae give numerals assigned to families in Table 2. *Vibrio* sp. and *B. mentericus* not included in averages of II and VI, respectively (see text).

Finally, it was possible to interpret the data in still another way. In Figure 4 all of the division rates of *Colpidium* grown for 10-day periods on experimental bacteria, as shown in Table 2, were plotted according to families. Since the history of *C. colpoda* grown on *P. fluorescens* was so much more uniform than that for *A. aerogenes*, it is more conservative and accurate to use only the data from the *P. fluorescens*—controlled experiments for this purpose. The available data show that the species in the Enterobacteriaceae supported the highest reproduction rates of *C. colpoda*, and Micrococcaceae and Bacillaceae the lowest division rates. The Rhizobiaceae, Pseudomonadaceae, and Bacteriaceae seem to form an intermediate group, as far as acting as food for *Colpidium* was concerned. Evidently, the Enterobacteriaceae could be said to contain the

best food organisms for *C. colpoda*, for, with the exception of the Bacteriaceae, which had only two representatives tested, the Enterobacteriaceae were the only family which had no toxic or decidedly unfavorable species. The Enterobacteriaceae were represented in this work entirely by Gram-negative species, all of which grew very quickly in pour plates. With the exception of the Bacteriaceae, which could not be grown in the usual dilutions in pour plates but supported a relatively high division rate of *Colpidium*, the vibrios, micrococci, sarcinae, and spore-formers all either did not grow or grew poorly in pour plates and also did not support very high productions of *Colpidium*. This appears to be related to the metabolism of the bacteria rather than to their abundance, since there were certainly large numbers of bacterial cells present.

A careful survey of the physiological properties of the above families in *Bergey's Manual of Determinative Bacteriology* (1939) gave no hint as to the reason for one family of bacteria being able to support a higher division rate of *C. colpoda* than another. It is generally known in bacteriology that *Escherichia*, *Aerobacter*, *Salmonella*, *Proteus*, and *Eberthella*, all belonging to the Enterobacteriaceae, have antigenic similarities. This fact

is of interest in connection with the homogeneity of the division rates of *C. colpoda* when grown on species of this family.

Since there are thousands of species of bacteria, only a small fraction of which can be readily obtained, much less grown in the laboratory, it is not likely that more than a small fraction will be tested as food for Protozoa. As mentioned earlier, type species of any genus were used whenever obtainable, so that as representative a sample as possible was tested. Of the thirty-one species of bacteria used, *C. colpoda* was maintained for a 10-day period on twenty-seven of them; therefore, it is safe to conclude that *C. colpoda* may be a polyphagous feeder and approaches euryphagy much more closely than it does stenophagy. Until it can be proved that Protozoa are selective feeders in nature and the synergic relations between bacteria are understood, any definite assignment of certain monoflorae as natural food for particular Protozoa must be withheld.

Hydrogen-ion concentration appeared to have little effect in the present experiments; in the large majority of experimental and control bacterial stock suspensions the pH remained very close to 6.4, while the total range was from 6.0 to 8.0. Only in the cases of the sarcinae, micrococci, and spore-formers were high pH readings taken; therefore, this may, in part, explain the poor showing of *Colpidium* grown on these bacteria. As the pH readings were taken only on the stock bacterial suspensions that were kept in a cold room, it is quite possible that profound pH changes took place in the 24 hours that the Protozoa were in the small 0.2-ml. drops. This may have been due to a germination of spores or to actual growth of the bacteria utilizing their own dead cells or the decomposition products thereof. Such a supposition is suggested by the fact that growth of all of the spore-formers which had encysted in the cold room was observed during the daily 24-hour experimental period.

With the exception of the extreme toxicity of *Ch. violaceum* little difficulty was experienced in maintaining *C. colpoda* on chromogenic bacteria. When *Ch. violaceum* was grown at 25° C., a 1/100 dilution⁸ of the concentrated suspension killed the *Colpidium* in less than three-quarters of an hour, while, when the same bacterium was grown at 37° C., much less of the deep-violet pigment was formed, and the Protozoa survived for about 8 hours. The other species in the same genus, *Ch. viscosum*, produced very little pigment and supported a good reproductive rate of Protozoa. Brilliantly pigmented stocks of both *Ser. marcescens* and *P. aeruginosa* were used with good results, although *Colpidium* died in drop cultures of the latter when they were allowed to run longer than 24 hours. The unpigmented strain of *Ser. plymouthensis* also supported one of the highest division rates of all bacteria used. Both species of *Flavobacterium* produced pigment, neither of which seemed to have an especially deleterious effect on *C. colpoda*. In the cases of *P. aeruginosa* and *F. synxanthum*, pigments of similar color were produced; and, although the action of both of these bacteria caused the veal infusion in the Kolle flasks to turn a fluorescent greenish yellow, this color must not have been taken up entirely by the solid medium, for the same color was imparted to the concentrated stock bacterial suspension. However, the stock used for protozoan growth was only roughly a 1/100 dilution of this concentrated stock, so that the pigment was perhaps below the threshold of its deleterious action when used for experimental purposes.

Although no record was kept of the size of individual *C. colpoda* grown on different bacteria, inspections indicated there was little, if any, relation between the dimensions

⁸ Colonies of *C. violaceum* did not grow in pour plates even in very low dilutions; for that reason the actual numerical concentration of this stock could not be determined.

of the individual and division rate. Some rapidly dividing cultures had relatively small individuals, while others regularly had forms which were quite large. The spore-formers supported very large colpidia, while *St. citreus* and the *P. fluorescens* controlled experiment of *Sar. lutea* produced very small ciliates with very low division rates in all three cases.

GENERAL DISCUSSION

Since Hetherington (1933, 1934) and Chatton and Chatton (1927a, b) are the major experimenters to have actually used *C. colpoda* in bacterial-protozoan studies, their work can be cited for comparison with this study. In general, this work is consistent with Hetherington's results, which it greatly extends. Chatton's brief reports on the action of the chromogenic bacteria on *C. colpoda* agrees with neither; however, since these men gave no indication of the actual concentrations of bacteria used, it is difficult to state categorically whether a real conflict exists. In 1934 Hetherington described somewhat vaguely the different effects that washed bacteria had on *C. colpoda*, in contrast with unwashed bacteria. Evidently results obtained in the present work are more comparable to his work with washed bacteria, for no agar substrate was carried into the bacterial suspensions used in these experiments. Pooling the bacteria studied in his 1933 work, which, according to Hetherington's own statement, were of doubtful purity because the *Colpidium* used was not sterile, with a few studied in his 1934 paper, a tabular comparison (Table 5) can be made between his results and those obtained in the present study. As the colpidia whose division rates are shown in the present paper were taken from cultures of *P. fluorescens* and of *A. aerogenes*, their division rates doubtless would differ from those of Hetherington, for in his 1933 work the *Colpidium* was taken from a "favorable medium (malted milk)." For these or other reasons, while certain similarities exist, there are as many differences.

Concerning the above effect of the source of the experimental Protozoa, it might be recalled that *C. colpoda* taken from *P. fluorescens* and grown on the same experimental bacteria as those taken from *A. aerogenes* differed statistically in their division rates. Those results are quite consistent with the host-selection principle of Hopkins (Allee and Park, 1939), which suggests that animals may at times select their habitat as a result of their previous experience. At any rate, with these Protozoa the past history of having lived in one bacterial suspension affected their performance in a suspension of another bacterium.

In 1933 Johnson found that very heavy inocula of *P. fluorescens* often killed the hypotrich *Oxytricha*, while lower concentrations of the bacterium proved to be excellent as a source of food. The use of supraoptimal inoculations of chromogenic bacteria may, in part, help to explain the disagreement between the present work and that of Chatton and Chatton (1927a, b) on *C. colpoda* and of Kidder and Stuart (1939) using *Colpoda*. All of these workers found *Ser. marcescens*, *P. fluorescens*, and *P. aeruginosa* to have deleterious effects on their experimental animals, while in the present work no such effect was observed. However, this paper, using *C. colpoda*, is in agreement with Kidder and Stuart (1939) on the action of *Ch. violaceum* on *Colpoda*, while the longer survival of *C. colpoda* on the less pigmented strain grown at 37° C. suggests that pigment actually caused the deleterious effect. Both the *Ch. viscosum* and the *Ser. plymouthensis* used in this work were unpigmented and supported a high division rate for *C. colpoda*. These

results are quite in agreement with the general observation that unpigmented strains of pigmented bacteria are better food for Protozoa than the pigmented.

Since Kidder and Stuart (1939) were able to grow *Colpoda* on bacteris suspended in distilled water, and in this work *C. colpoda* was grown on bacteria suspended in artificial pond water as well as in the double distilled water which was used in preliminary experiments, it is surprising that Hetherington (1933) insists that "no bacterium was discovered which, when suspended in fresh water (Peters' medium), would support growth

TABLE 5
TABULAR COMPARISON WITH HETHERINGTON'S DATA

BACTERIA USED	GROWTH OR DIVISION RATE OF <i>C. colpoda</i>		
	Hetherington's Results*	Present Work (Av. of Four Lines for 10 Days)	
		<i>P. fluorescens</i>	<i>A. aerogenes</i>
<i>Al. fecalis</i>	Fair	1.54	0.88
<i>P. aeruginosa</i>	Fair	1.07	1.84
<i>P. fluorescens</i>	Large	2.75	3.00
<i>M. conglomeratus</i>	1.99	1.34	0.73
<i>E. coli</i>	Toxic	2.14	1.22
<i>A. aerogenes</i>	4.33	2.93	1.82
	2.74		
	Very large		
<i>A. cloacae</i>	Fair	2.38	1.51
<i>Pr. vulgaris</i>	Large	2.73	1.25
<i>B. subtilis</i>	Large	0.08	0.33
<i>B. mesentericus</i> (unwashed)	2.70	Survived 1 day	0.20

* Verbal descriptions of growth were taken from Hetherington's 1933 paper, in which the length of the experiments was not mentioned; numerical descriptions from his 1934 paper indicate 7-day averages.

of *C. colpoda* for more than a few generations." As mentioned earlier (see "Material and Methods"), stock protozoan cultures were kept regularly for 1 month. At widely fluctuating room temperatures old stocks were kept for 3 months, and at $25^{\circ}5 \pm 0^{\circ}5$ C. they ran for 6 months, while in a cold room at 6° C., *C. colpoda* in an *A. aerogenes* suspension has been maintained for 10 months. It must, therefore, be concluded either that the methods used in this work and by Kidder and Stuart (1939) were imperfect or that Hetherington has not used optimal inocula of food bacteria or that his preparation of Peters' medium was somewhat toxic.

There are certain strong suggestions (Table 3) that different species of bacteria be-

longing to the same genus may be quite different in their ability to support protozoan growth. It is too early to state definitely that these differences were any more than tendencies; but, when it is also seen that Cleveland (1928), Hetherington (1934), Kidder and Stuart (1939), and Leslie (1940), using widely diverse species of Protozoa, all found indications that strains of the same bacterial species had different growth-promoting effects when used as food for the several experimental animals, it is not surprising that similar differences may exist among species of bacteria in the same genus.

The science of bacteriology has solved many of its technical problems in such a way that bacteriologists all over the world can make use of the same techniques and, therefore, obtain comparable results. Many of the techniques used in bacteriology can be carried over into the study of Protozoa that will live on nonliving-particulate or nonparticulate media. However, under present-day culture conditions at least, many holozoic Protozoa must have living food; for that reason the old problem of method again arises. Usually, bacterial cultures are seeded with large inocula; and, because of the rapid division rate of the bacteria, the standard length for which they are allowed to grow is generally 24 hours. The same techniques will not give comparable results with Protozoa grown on different bacteria unless a gross inoculum of Protozoa in mass cultures is used, for it is usually impossible in 24 hours to obtain enough animals for a sampling count if more than a few cubic centimeters of bacterial suspension are used. Small samples taken over a period of time from mass cultures do give valuable information about the shape and amplitude of the growth curve of numbers in the growing population; but, unless the same-sized inoculum, the same amount of food, the same volume, and the same time lapse are used, very different results will be obtained (Hall, 1941a). Inconsistent use of mass culturing as a technique has led to the present somewhat confused state of experimental work in this field.

Unfortunately, the growth of Protozoa in isolation cultures using bacteriological methods plus daily counts of Protozoa is highly laborious, especially when bacteria-free protozoans are grown on a monoflora. However, for the reasons cited earlier, it has obvious advantages over the mass-culture technique for establishing simple accurate relationships between Protozoa and species of bacteria. The erratic results obtained by Hetherington (1934) for *C. colpoda* grown on *A. aerogenes* in mass culture and the same type of results obtained in isolation culture for the same organisms in this paper indicate that, for some organisms at least, many readings must be made to obtain merely a comprehensive description of existing relationships. These extra readings are needed regardless of whether mass or isolation cultures are to be used. Present experience indicates that fifteen to twenty entirely separate determinations should suffice.

Protozoa growing in mass cultures may approach closely the condition in nature, while growing Protozoa in isolation cultures is an artificial method which can be justified since it allows the analysis of many of the relations found in the larger complex. Mass cultures produce so many individuals that statistics are quite applicable; but much significance is lost, for only surviving individuals are used to describe what is happening within the population. On the other hand, many readings using isolation cultures are necessary for adequate tests of statistical significance. However, so much more accuracy is gained by the second method that for certain types of study the extra effort is warranted.

Several authors (Cleveland, 1928; Hetherington, 1934; Kidder and Stuart, 1939; Leslie, 1940) have pointed out that different strains of bacteria of the same species affect the division rates of Protozoa in different ways. For a study of such happenings the isolation-culture method is useful, because the 24-hour counts allow the interaction be-

tween bacteria and Protozoa to be studied in much the same way that other physiological tests are used in the study of bacteria. It is quite possible that subtle differences in different strains of bacterial species may be explored by growing Protozoa on them, especially if relatively standardized and stable Protozoa can be found. In this case, Protozoa would become indicators for the use of the bacteriologist; but care must be taken, for Hetherington (1934) found that Protozoa react quite differently to bacteria grown in bacterial broths, in contrast with those suspended in balanced salt solutions. The increase in the division rate found in his work when *Colpidium* was grown in bacterial suspensions in broths may have been due to the partial utilization of the broth by the protozoan itself or to the added food supplied by an increased number of bacteria growing in the broth.

It is commonly known that certain domesticated herbivores cannot be successfully raised on poor fodder containing weeds of little nutritional value. As Allee (1941) has pointed out, the early investigators in protozoölogy may have inadvertently included weeds in their "hay-tea" cultures. Possibly such weeds might have affected the Protozoa grown in hay-tea; however, the present analysis has gone a step further, for now a less obscure interaction between Protozoa and their food has been established. Certain bacteria, because of their excellency as food, might be called "microfodder," while others, of low nutritional value, are nothing more than weeds, as far as the polyphagous "micro-herbivore" *C. colpoda* is concerned.

SUMMARY

1. A controlled isolation-culture technique was used to study the bacterial-protozoan relationships between thirty-one species of Eubacteriales and *C. colpoda*.
2. In all but a few cases the experimental bacteria caused significant differences in the division rate of *Colpidium*, as compared with simultaneous tests of its division rate on control bacteria.
3. There are indications that *C. colpoda* is a polyphagous bacterial feeder, since it was maintained for a 10-day period in suspensions of twenty-seven out of thirty-one bacteria used.
4. *Colpidium* grown on *P. fluorescens* differed significantly in division rate from that grown on *A. aerogenes*.
5. The source of experimental animals was important, for *Colpidium* taken from *P. fluorescens* and from *A. aerogenes* and placed on suspensions of the same experimental bacteria react significantly differently as to division rates and survival time.
6. The family Enterobacteriaceae supported the highest division rate of *C. colpoda* out of six families, while the Bacillaceae supported the lowest.
7. It is not yet apparent what physiological property or properties of these Gram-negative organisms common to the whole family Enterobacteriaceae makes its members especially good food organisms for *C. colpoda*.
8. From preliminary statistical tests it appears that different species of bacteria belonging to the same genus have significantly different growth-promoting powers when used as food for *C. colpoda*.

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